# Studies on *Oxyspirura mansoni*, the Tropical Eyeworm of Poultry. II. Life History

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THIS PAPER, the second in a series, presents the results of a study made in Hawaii of the life history of *Oxyspirura mansoni*, the tropical eyeworm of poultry. This nematode has been shown by previous investigators to possess a digenetic life cycle in which it utilizes as the definitive host many species of domestic and wild birds and, as the secondary host, a single species of burrowing cockroach, *Pycnoscelus surinamensis*.

The parasite is widely distributed in the warmer parts of the world. It has been reported from Brazil (de Magalhaes, 1888; Almeida, 1933), China (Cobbold, 1880), Indo-China (Fielding, 1928*b*), Mauritius (Emmerez and Megnin, 1901), Reunion (Ozoux, 1910), British West Indies (Hutson, 1943), Formosa (Kobayashi, 1927), British and Dutch East Indies (Picard, 1929; Fielding, 1928*b*), Florida and Jamaica (Ransom, 1904), Hawaii (Wilcox and McClelland, 1913), Guam (Fielding, 1928*b*), and Samoa (Alicata, personal communication). Its near relative (or synonym), *Oxyspirura parvovum*, has been reported from Australia by Dodd (1909).

Apparently Manson's eyeworm has approximately the same circumtropical distribution as its intermediate host (reviewed in Schwabe, 1949). Neither parasite nor host is known to exist in Hawaii at altitudes exceeding 3,000 feet.

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The eyeworm is considered important economically in many tropical and subtropical areas. The roach is objectionable not only in that it serves as the vector for the eyeworm, but also because it reportedly damages both roots and bark of certain ornamental and crop plants (reviewed by Schwabe, 1949). Previous to this study the development of the larval and adult stages of this parasite was not well known.

## MATERIALS AND METHODS

Surinam roaches in all stages of development were easily collected from soil beneath chicken houses and in chicken yards. Because the incidence of natural infection with the parasite among such roaches approached 100 per cent, only laboratory-raised nymphs were employed in the experimental infections.

Adult *Pycnoscelus surinamensis* females were housed individually in 4-inch glass stacking dishes. A 3-inch disk of filter paper was placed in each dish to provide a hiding place for the roaches during the daytime. Nymphs, isolated at birth, were housed together in the same manner as the adults. The roaches thrived on a diet of whole-wheat bread and water.

Embryonated eggs for infecting laboratory-raised roaches were obtained by macerating gravid female worms. This procedure proved more satisfactory than the involved method of concentration and separation of embryonated eggs from the feces of infected birds.

The gravid worms were taken from the eyes of infected chickens obtained from poultry farms in Manoa Valley and Waialae, Hono-

lulu. The parasites were removed from the eyes of the birds by holding the head rigid and inserting the tips of a pair of dull-pointed forceps beneath the nictitating membrane. Worms thus obtained usually survived for at least 24 hours in physiological saline at room temperature.

Young nymphs to be infected were first isolated without food or water in clean stacking dishes for at least 48 hours. Nymphs which were dead or enfeebled after this treatment were removed. Gravid female eyeworms were macerated in a drop of water to free the eggs from the vagina and uteri. The entire mass was then soaked up with a small bread crumb. The starved nymphs rapidly ate the moistened bread. It was later found that the nymphs would just as readily consume living female worms so this method of infection was adopted.

A roach to be examined was pinned, dorsal side downward, on a paraffin block. The head was severed with a sharp scalpel and the posterior abdominal segment was teased from the remainder of the abdomen with fine dissecting needles. The rectum remained attached to this segment and the entire alimentary tract was withdrawn in this manner. The alimentary tract and the remainder of the roach were each placed in a few drops of physiological saline solution on separate clean glass slides. Free third-stage larvae, if present, could, with the unaided eye, then be seen wandering about on the slide. Encysted second- and third-stage larvae were readily observed entangled in the Malpighian tubules or attached elsewhere along the alimentary canal, particularly in the region of the rectum.

To observe the late first-stage larvae, the abdominal fat was dissected away from the body wall, placed in a drop of physiological saline solution, and examined beneath a cover slip with the aid of a compound microscope.

In examinations for early first-stage larvae, the crop and esophagus were severed from the remainder of the alimentary canal, teased apart, and examined as a wet mount with a compound microscope. This procedure was repeated on separate slides for the midgut and hindgut.

First-stage larvae were examined as live wet mounts or were stained vitally with methylene blue or fixed in Bouin's solution. Second- and third-stage larvae were examined alive by compressing the cyst beneath a cover slip or by freeing the larvae from the cysts and fixing them in Bouin's solution. Fourth-stage larvae were killed in Bouin's solution and examined as wet mounts.

When living larvae were too active for study they were anesthetized by a crystal of chloral hydrate introduced beneath the cover slip.

The chickens used in these experiments were all hybrid stock (Rhode Island Red × New Hampshire Red) obtained at the age of 2 weeks from the Department of Poultry Husbandry, University of Hawaii. They were housed on wire and fed commercial growing mash.

Third-stage larvae were first obtained in the laboratory by the dissection of large numbers of infected roaches. Inasmuch as this consumed so much time and the larvae were not obtained in the numbers desired, a more satisfactory method was devised. It was observed that when infected roaches were torn apart and placed in physiological saline solution, heated to approximately 37° C., the larvae immediately began to migrate from the tissues of the roaches and to settle to the bottom of the container. This behavior of the larvae suggested the use of the Baermann apparatus, with which an ample supply of infective larvae was readily obtained.

## THE ADULT EYEWORM

The sexually mature worms are found beneath the nictitating membranes and in the conjunctival sacs and naso-lacrimal ducts of domestic chickens, ducks, and a number of other wild and domesticated birds.

### *Anatomy of the adult eyeworm*

The adult *Oxyspirura mansoni* is a slender,

TABLE 1

REPORTED MEASUREMENTS OF ADULT FEMALE EYEWORMS

| MEASUREMENT | SCHWABE (Oahu, T.H.) 1948–49 | RANSOM (Florida) 1904 | MAGALHAES (Brazil) 1888 | SWEET (Australia) 1910* | FIELDING (Australia) 1928* |
|---|---|---|---|---|---|
| Length† | 15–17 | 12–18 | 16.2‡ | 13.5–20 | 15.5–17 |
| Width (ant. end) | 69.1–75 | 50 | | | |
| Width (max.) | 369–538 | 400–430 | | 270–390 | 270–420 |
| Width (at anus) | 92‡ | 90–100 | | | |
| Esophagus length† | 1.55‡ | 1.5 | | | |
| Esophagus width (post. end) | 107‡ | 80–100 | | | |
| Intestine width (at esophagus) | 61.5‡ | | | | |
| Intestine width (max.) | 77–100 | 100 | | | |
| Anus to post. end | 446–477 | 400–530 | 400–530 | 390–440 | 364–509 |
| Protostom length | 15.4–22 | 15–25 | | | |
| Protostom width | 35.7‡ | 25–30 | | | |
| Mesostom length | 22–31 | 25–30 | | | |
| Mesostom width | 17.3‡ | 20–25 | | | |
| Excretory pore from ant. end | 410‡ | 350–400 | | | |
| Nerve ring from ant. end | 232‡ | 250 | | 220–300 | 270–320 |
| Vulva diameter | 46–61 | 40–50 | | | |
| Vulva to post. end† | 1.23–1.31 | 1–1.4 | 1–1.33 | 0.78–1.07 | 0.91–1.55 |
| Vagina length† | 2 | | | | |
| Vagina width (near uteri) | 57.1‡ | 50 | | | |
| Vagina width (near vulva) | 36‡ | | | | |
| Uteri width | 123–146 | 100 | | | |
| Ovary width | 22–30 | | | | |

*_Oxyspirura parvovum_.

†These measurements in millimeters; all others in microns.

‡Average measurements (10 specimens).

TABLE 2

REPORTED MEASUREMENTS OF ADULT MALE EYEWORMS

| MEASUREMENTS | SCHWABE (Oahu, T.H.) 1948–49* | RANSOM (Florida) 1904 | SWEET (Australia) 1910† | FIELDING (Australia) 1928† |
|---|---|---|---|---|
| Length‡ | 9.2–13 | 12–14 | 9.2–14.5 | 8.2–15.47 |
| Width (ant. end) | 75 | 50 | | 74 |
| Width (max.) | 339 | 200–350 | 260–330 | 254–327 |
| Width (at cloaca) | 143 | 65–150 | | 136 |
| Esophagus length‡ | 1.37 | | | |
| Esophagus width (post. end) | 107 | | | |
| Intestine width (max.) | 111 | | | |
| Buccal capsule length | 53.5 | | | |
| Excretory pore from ant. end | 410 | | | |
| Nerve ring from ant. end | 285 | | | |
| Cloaca to post. end | 285 | 320–400 | | 253–390 |
| Long spicule length‡ | 4.15 | | | 3.64–4.55 |
| Short spicule length | 232 | | | 214–235 |

*Average measurements (10 specimens).

†_Oxyspirura parvovum_.

‡These measurements in millimeters; all others in microns.

FIG. 1. Photomicrograph showing the anterior end of an adult *Oxyspirura mansoni*.

white, thread-like worm attenuated at both the anterior and posterior ends. Adults studied in the laboratory measured from 9 to 17 mm. in length (Tables 1 and 2). The posterior end is more slender and in the male is curved ventrad. The thin, transparent, outer cuticle is smooth; neither transverse nor longitudinal striations were discernible on worms studied. Magalhaes (1888) reported fine transverse striations; these were not apparent to Emmerez and Megnin (1901) or to Ransom (1904).

The following papillae are common to both sexes: a small cervical papilla with a short hair-like process located on each side of the body approximately 440$\mu$ from the anterior end; a pair of small latero-caudal papillae near the posterior extremity of the tail; six minute oral papillae; and four large sublateral cephalic amphids. In the male, six pairs of papillae

FIG. 2. Photomicrograph showing the posterior end of an adult male *Oxyspirura mansoni*.

surround the cloacal opening, four pairs preanal and two pairs postanal.

The muscle arrangement is of the polymyarian type, with a fan-like arrangement of muscle fibers in the anal region of both the male and the female, attaching the rectum to the dorsal wall.

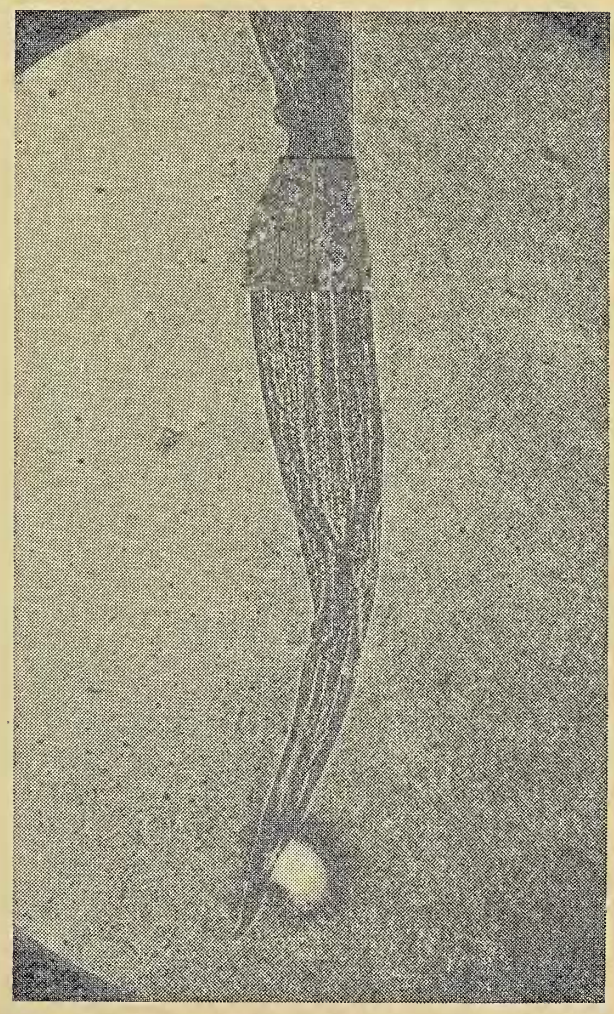

FIG. 3. Photomicrograph showing the posterior end of an adult female *Oxyspirura mansoni*.

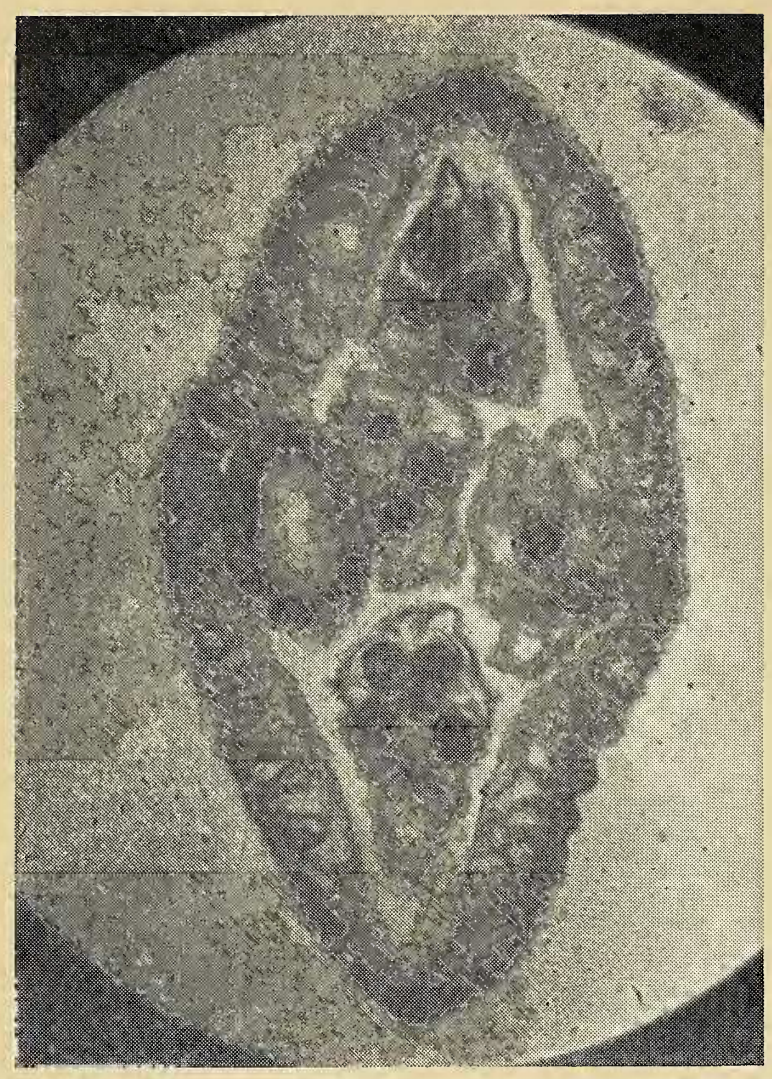

FIG. 4. Photomicrograph of median cross section through an adult female eyeworm.

The cuticular buccal capsule is six-lobed and, as is characteristic of the Thelaziidae, is divided into two chambers, the anterior protostom and the posterior mesostom. The esophagus is of the typical spirurid type and is approximately 1.5 mm. in length. It is distinctly divided into a short anterior muscular portion and a posterior glandular portion. The lumen is restricted and triradiate when viewed in cross section. A well-developed esophageal-intestinal valve separates the esophagus from the intestine.

The thin-walled intestine extends almost the entire length of the body, varying only slightly in diameter, and joins the esophagus anteriorly and the rectum posteriorly. The intestinal wall is composed of a single layer of short, ciliated columnar cells. The rectum is thick-walled and muscular and terminates at the ventral anus in the case of the female. In the male, the ejaculatory duct joins the rectum ventrally to form the cloaca.

The excretory system is of the simple H or oxyuroid type. From the ventral pore the common excretory duct extends dorsally through the large excretory cell and divides on the ventral side of the esophagus to join the two lateral excretory canals. These lie embedded in the lateral lines of the body wall. The lateral lines, which extend almost the entire length of the body, are, apparently, each a single large multi-nucleate cell. The lateral canals terminate posteriorly at the laterocaudal papillae.

A nerve ring surrounds the esophagus, cephalic of the excretory pore. In association with it are four large ganglia, two dorsal and two ventral, and a number of smaller cells. A dorsal and a ventral nerve cord extend posteriorly the length of the body, and several smaller nerve fibers extend into the cephalic region.

The mature male possesses two spicules of unequal length, both of which are cuticular in nature, transversely striated, and hollow. The shorter, which is "trough-shaped," measures about 200$\mu$ in length by 30$\mu$ in maximum width, is only slightly protrusible, and acts primarily as a guide for the longer, more slender spicule. The long spicule is 3 to 4.2 mm. in length by 10$\mu$ in maximum width and is capable of being protruded from the cloaca almost its entire length. A short muscular ejaculatory duct extends anteriorly from the cloaca and terminates in a thin-walled seminal vesicle, an uncoiled organ extending over half the length of the body. The single long, coiled testis fills the remainder of the body cavity.

In the female the vulva, which measures 46 to 51$\mu$ in diameter, is located ventrally in


FIG. 5. The short, "trough-shaped" spicule of the adult male eyeworm.

the posterior half of the body. It is surrounded by a cuticular ring of highly refractile material. The vagina extends anteriorly from the vulva and is divisible into two parts, by reason of the thickness of the wall. The proximal portion is nearly 150 $\mu$ in length and 35 $\mu$ in diameter. The distal thicker-walled portion is approximately 60 $\mu$ in diameter. The vagina branches to form the two thin-walled uteri. These extend forward to the posterior region of the esophagus, where they reflex and extend back to the region of the vulva. The diameter of the uteri decreases to 22–30 $\mu$ to form the two ovaries which lie as a coiled mass in the posterior portion of the body cavity.

## HOSTS OF THE EYEWORM

Nematodes inhabiting the eyes of birds have been reported from a number of species. Most of these parasites are members of the genera *Thelazia* and *Ceratospira*, or are species of the genus *Oxyspirura* other than *O. mansoni*.

The hosts of only those eyeworms which have been definitely identified as *Oxyspirura mansoni* (or *parvovum*) are recorded here.

*Definitive hosts*

Manson's eyeworm has been reported to occur naturally in the following birds: domestic chicken (*Gallus domesticus*) (Cobbold, 1880); turkey (*Meleagris gallopava*) (Cram, 1927); peafowl (*Pavo cristatus*) (Magalhaes, 1888); English sparrow (*Passer domesticus*) (Illingworth, 1931; Alicata, 1947); mynah bird (*Acridotheris tristis*) (Alicata, 1947); Chinese dove (*Streptopelia chinensis*) (Alicata, 1947; Schwartz and Schwartz, 1949); Japanese quail (*Coturnix coturnix japonica*) (Schwartz and Schwartz, 1949); pheasant (*Phasianus torquatus torquatus* and *P. vesicolor vesicolor*) (Schwartz and Schwartz, 1949).

Through the cooperation of Paul Breese, Director, the birds in the Honolulu Zoo are being examined for the presence of *Oxyspirura mansoni*. This survey is not complete as yet, but the following new hosts may be recorded: the great Argus pheasant (*Argusianus argus argus*) and the Siamese fireback pheasant (*Diardigallus diardi*).

*Role of the natural reservoir hosts in the spread of the parasite in Hawaii*

During the course of this investigation mynah birds, English sparrows, and Chinese doves have been trapped on the University campus and have been found to harbor Manson's eyeworm. Similar observations have been made by other investigators in other parts of Hawaii (Illingworth, 1931; Alicata, 1947; Schwartz and Schwartz, 1949; Tanada, personal communication).

TABLE 3
NUMBERS OF INFECTIVE LARVAE PER ROACH
(100 SURINAM ROACHES EXAMINED AT EACH LOCALITY)

| STAGE | RANGE | MIYATA POULTRY FARM, WAIALAE, OAHU | UNIVERSITY FARM, MANOA VALLEY, OAHU* | WAIKIKI, OAHU† |
|---|---|---|---|---|
| Adult roaches | Max. | 36 | 3 | None |
| | Min. | 16 | 0 | |
| | Ave. | 23 | | |
| Nymphs (Final instar) | Max. | 34 | 2 | None |
| | Min. | 1 | 0 | |
| | Ave. | 3 | | |
| Young nymphs | Max. | 6 | 0 | None |
| | Min. | 0 | 0 | |

*Roach population low (daily removal of manure, weekly chlordan spray).
†Approximately 4 miles from nearest poultry farm.

Since these commonly infected birds are widespread and numerous throughout the Hawaiian Islands, it seemed desirable to ascertain the role they play in the dissemination of the parasite. To determine this, the degree of infestation of Surinam roaches was established at (1) a heavily infested poultry farm in Waialae, Honolulu, Oahu; (2) a relatively clean poultry yard in Manoa Valley, Honolulu; and (3) a residential area in Waikiki, Honolulu. In all three places roaches were numerous.

As shown in Table 3 the roaches from (1) harbored a large number of infective eyeworm larvae per roach. At (2), where low infestation of the chickens resulted from strict sanitation and frequent removal of the manure, but where large numbers of wild birds gathered to feed, the number of larvae per roach was very low. At (3), an area at some distance from any poultry farm, but where wild birds were also very numerous, no larvae were found in the many roaches examined.

Inasmuch as domestic fowl and wild birds are the only sources of infection of the local roaches these data indicate that the local wild birds are of little importance as reservoir hosts in the dissemination of the eyeworm population. Apparently their feces are too scattered to be eaten to any great extent by the roaches.

*Mammals as hosts of Manson's eyeworm*

In no case has *Oxyspirura mansoni* been known to occur naturally in the eyes of a mammal. Fielding (1927) found that infective eyeworm larvae placed in the eyes of guinea pigs would develop to maturity.

To check Fielding's observations on another mammal, I obtained several white rats. Each of these was forcibly fed four infected roaches. The next day the eyes were anesthetized with a 5 per cent solution of butyn and examined carefully for eyeworm larvae. No larvae were found.

Approximately 30 infective larvae were then introduced by pipette into the mouth of one of the rats. On examination of the eyes 24 hours later, no larvae were found.

Two rats then received approximately five eyeworm larvae per eye. One was examined after 10 days and larvae were seen in both eyes. The rat was necropsied and the worms were removed. They had molted to the fourth stage and the reproductive organs were developing normally. The second rat was killed 25 days after the larvae were placed in its eyes. Adult male and female worms were recovered from both eyes.

## LIFE HISTORY OF THE PARASITE

Apparently little effort had been made before about 1927 to ascertain the life cycle of *Oxyspirura mansoni*. Previously Emmerez and Megnin (1901), and Emmerez (1918), Ransom (1904), and Ozoux (1910) had attempted to transmit the infection directly from one chicken to another with embryonated eggs and/or first-stage larvae; but they carried the work no further when their efforts were unsuccessful.

Kobayashi (1927) in Formosa and Sanders (1928) in Florida found that an intermediate host, which they identified as the cockroach *Pycnoscelus surinamensis* Linn., was essential for the completion of the life cycle of the parasite. Previously Fielding (1926) had shown the same roach to be the intermediate host of the Australian eyeworm of poultry, *Oxyspirura parvovum* Sweet. Fielding (1927 and 1928*a*) studied the developmental anatomy of the Australian eyeworm, but because of his uncertainty as to whether the species was *O. mansoni* or *O. parvovum*, he referred to it simply as the eyeworm of poultry in his latter paper. The validity of *O. parvovum* as a species was questioned by Tryon (1926). This lack of clarity as to species, together with the fact that Fielding's drawings were not identifiable with the material found in Hawaii, led me to investigate the life history of Manson's eyeworm under Hawaiian conditions.

*Life cycle in general*

The eggs of the parasite are laid by the

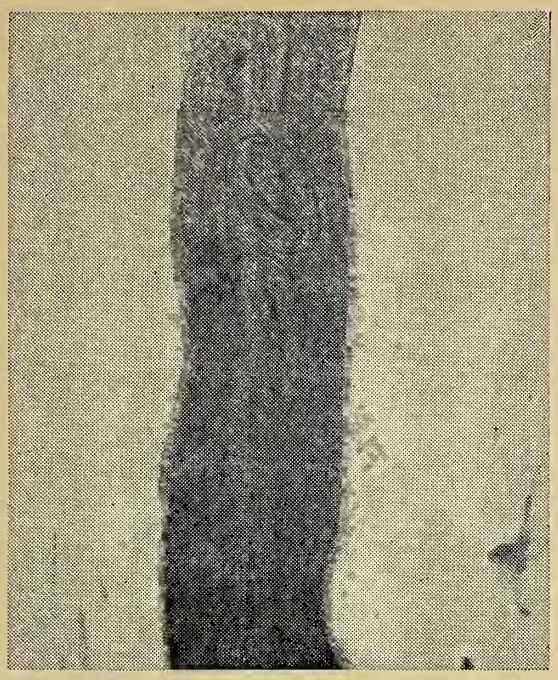

FIG. 6. Photomicrograph of the vagina of a gravid female eyeworm showing embryonated eggs.

adult females in the eyes of the definitive host. They are washed down the naso-lacrimal ducts with the eye fluid into the mouth, swallowed, and finally passed out with the excrement. The intermediate host is infected by eating embryonated eggs or first-stage larvae in the feces of infected birds. After a developmental period of approximately 51 days in the roach, the larvae are infective to the definitive host. They gain entrance when the host roach is consumed by a suitable bird. The larvae seldom pass farther than the crop of the definitive host (Fielding, 1926), where they migrate from the tissues of the roach under stimulation of heat and moisture.

The infective larvae then crawl up the esophagus, reach the roof of the mouth, and gain entrance to the eyes through the naso-lacrimal ducts. During this study worms have been observed in the eyes of a chicken within 5 minutes after the ingestion of an infected roach.

*The eyeworm eggs*

During the course of this study several attempts were made to recover embryonated eggs from the eyes of infected birds. Fluid was removed from the eye with a pipette and examined beneath the microscope, but in no case were eggs observed. However, they were recovered in varying quantities from the crop, intestinal contents, and feces of infected birds. The eggs were elliptical in shape, had a shell thickness of 1 to 1.5$\mu$, and were approximately the same size as those measured in the

TABLE 4

REPORTED MEASUREMENTS OF EYEWORM EGGS (ALL MEASUREMENTS IN MICRONS)

| STAGE | RANGE | SCHWABE (Oahu, T.H.) 1948–49 | RANSOM (Florida) 1904 | SWEET (Australia) 1910* | FIELDING (Australia) 1928† | FIELDING (Australia) 1928* |
|---|---|---|---|---|---|---|
| Embryonated eggs from feces | Max. | 60x45 | | | | |
| | Min. | 42x23 | | | | |
| | Ave. | 53x40 | | | | |
| Embryonated eggs from vagina and uteri | Max. | 50x28.5 | 65x45 | 45x30 | | |
| | Min. | 35.7x17.8 | 50x40 | 33x25 | | |
| | Ave. | 41.6x23.7 | | | 43x31 | 41x30 |
| Shelled, segmented eggs from uteri | Max. | 42.9x32 | | | | |
| | Min. | 28.4x14.3 | | | | |
| | Ave. | 36.7x22.9 | | | | |
| Unshelled, non-segmented eggs from uteri | Max. | 28.25x10.7 | | | | 28x14 |
| | Min. | 21.6x7.2 | | | | 24x12 |
| | Ave. | 23.4x9.5 | 24x12 | | | |

*O. *parvovum*.
†Old fixed *O. mansoni* eggs.

vagina of gravid female worms (Table 4). The shell had a smooth surface with no discernible markings.

Ransom (1904) obtained embryonated eggs from the uteri of gravid worms. These were placed in "salt solution" and were reported to have hatched in 3 days. The larvae obtained were dead, or were feeble and soon died. Sanders (1928) employed a similar technique, substituting distilled water for the saline solution, and found that embryonated eggs from gravid females hatched in 3 days.

Fielding (1927) succeeded in hatching living larvae in the following media: 0.9 per cent NaCl, 1.4 per cent NaCl, other physiological solutions, 1 per cent citrated fowl blood, moistened earth, fowl feces (plus sand and saline), sterile fowl feces (plus saline and charcoal), moistened bread. Hutson (1943) was unsuccessful in hatching eggs in distilled water but obtained living first-stage larvae from cultures of distilled water plus fowl feces. Upon introduction into the body cavity of a roach these larvae developed normally.

It seemed desirable to determine, if possible, whether eyeworm eggs hatch more readily in the digestive tract of the intermediate host or in fowl feces. Attempts were made to obtain mature eggs from the crops of infected chickens. (Some embryonated eggs obtained from the uteri of gravid worms may not be fully matured. Such eggs in other species of nematodes have been known to "hatch" abnormally in artificial media.) Although eggs were usually present in the crop, they could not be separated in sufficient quantity for the experiment. This made it necessary to obtain embryonated eggs from macerated female worms. To avoid as far as possible the selection of immature eggs, special care was taken to collect by use of a fine pipette only the largest eggs.

These were placed in three dishes containing (1) physiological saline solution, (2) physiological saline solution plus sterilized fowl feces, and (3) physiological saline solution plus the macerated alimentary tracts of several Surinam roaches. None of the eggs had hatched by the end of the second day, but quiescent or feeble larvae were observed in each of the three cultures on the third day. None of the larvae survived. Although additional work is indicated, apparently, under the conditions of this experiment, the medium had no appreciable effect upon the rapidity with which eyeworm eggs hatched.

Larvae observed both in the artificial media and in the alimentary tract of the intermediate host apparently hatched in the following manner. (The terminology used is based on the description of the spirurid egg by Christenson in Chitwood *et al.*, 1940.) The chitinous middle layer of the shell separated from the inner vitelline membrane at either or both poles. The region of the poles then became thinner, producing polar operculations marked by ill-defined sub-terminal lines of fracture. One or both caps either broke off at the line of fracture or dissolved, leaving a barrel-shaped shell, open at either or both ends. The embryo then freed itself from the thin vitelline membrane and squeezed through the polar opening.

Similar observations have been made by Ransom (1904), Sanders (1928), and Fielding (1927).

*Development in the intermediate host*

Eggs were obtained from gravid female worms and were fed to young laboratory-raised roach nymphs. One nymph was dissected 24 hours after feeding, and numerous embryonated and non-embryonated eggs were found in the crop. No eggs or egg shells were found in the feces of the nymphs examined at this time.

At 48 hours, several first-stage larvae were found free in the lumen of the crop and anterior midgut, and many others were observed in the process of hatching. The empty shells were barrel-shaped, which suggested that they hatched by splitting off one or both polar caps, as previously described. Several of the larvae were rather firmly attached to cellular

debris by the tip of the tail. Embryonated eggs were found in the midgut until the seventh day, and numerous larvae were present until the ninth day. A single larva was found in the midgut on the eighteenth day. It was almost double the size of those seen there previously.

First-stage larvae were observed for the first time in the body cavity on the eighth day, and by the tenth day many were seen. Some were wandering freely in the cavity, but most were found in the adipose tissue lining the abdominal wall. Apparently they migrated through the wall of the midgut, and after wandering in the body cavity for a short time, burrowed into the abdominal fat. The larvae became lethargic at this stage and were extremely difficult to extricate. They grew only slightly in length, broadened considerably, lost their long, pointed tails, and assumed a more-or-less club-shaped appearance because of the enlargement of the several large rectal cells.

Incomplete encystment actually occurred in this stage in some instances, with the ill-defined cyst measuring approximately 0.3 mm. by 0.5 mm. Thereafter little change took place until about the seventeenth day when larvae measuring 250$\mu$ to 320$\mu$ in length and with the cuticular sheath loosened at both the anterior and posterior ends were observed. Larvae in this pre-molting condition were seen until the twenty-fifth day, at which time a second-stage larva which measured 990$\mu$ in length was observed.

Complete encystment occurred in the second stage of larval development. The cysts were nearly spherical in shape and measured approximately 0.8 mm. by 0.8 mm. They were found attached to the alimentary tract, particularly around the rectum and entangled in the Malpighian tubules. The cysts were thin-walled and transparent. The wall, apparently of loose connective tissue, consisted of a gelatinous matrix and a few scattered cells. The wall was richly tracheated, which constituted evidence that the cyst was formed by the cockroach and not secreted by the parasite. The cysts were filled with fluid and the coiled larvae were able to move about freely in them.

On the thirty-third day encysted second-stage larvae measuring 1.8 mm. to 1.85 mm. were observed; those on the fortieth day had attained a length of 2.6 mm. to 4.5 mm. On the forty-fifth day a second-stage larva 6.62 mm. in length was observed in the process of molting. Ecdysis evidently takes place between the forty-fifth and fiftieth days, at which time the last molting larvae were seen.

Many of the parasites were able to free themselves from their cysts through their increased activity during the period of ecdysis, and on the fifty-first day numerous third-stage larvae were found, some encysted, but most wandering freely in the body cavity. These early third-stage larvae measured from 7.4 mm. to 8.3 mm. in length and were extremely active.

Whether or not those larvae which were unable to free themselves during the molting period eventually escape from the cyst could not be ascertained.

Upon the attainment of the third stage, the larvae were infective to the definitive host, as was shown by their appearance in the eyes of chickens within 5 minutes after they were introduced into the mouth by pipette.

*Development in the definitive host*

Large numbers of infective larvae were obtained from roaches by use of the Baermann apparatus. They were introduced by pipette into the mouths of 4-week-old chicks. The larvae migrated up the naso-lacrimal ducts and were observed to enter the eyes several minutes after they were introduced. Chicks were killed every 2 days, and the worms were removed from the eyes and examined microscopically.

On the second day the larvae appeared much the same as they did in the cockroach. On the fourth day several third-stage larvae in the process of molting were observed.

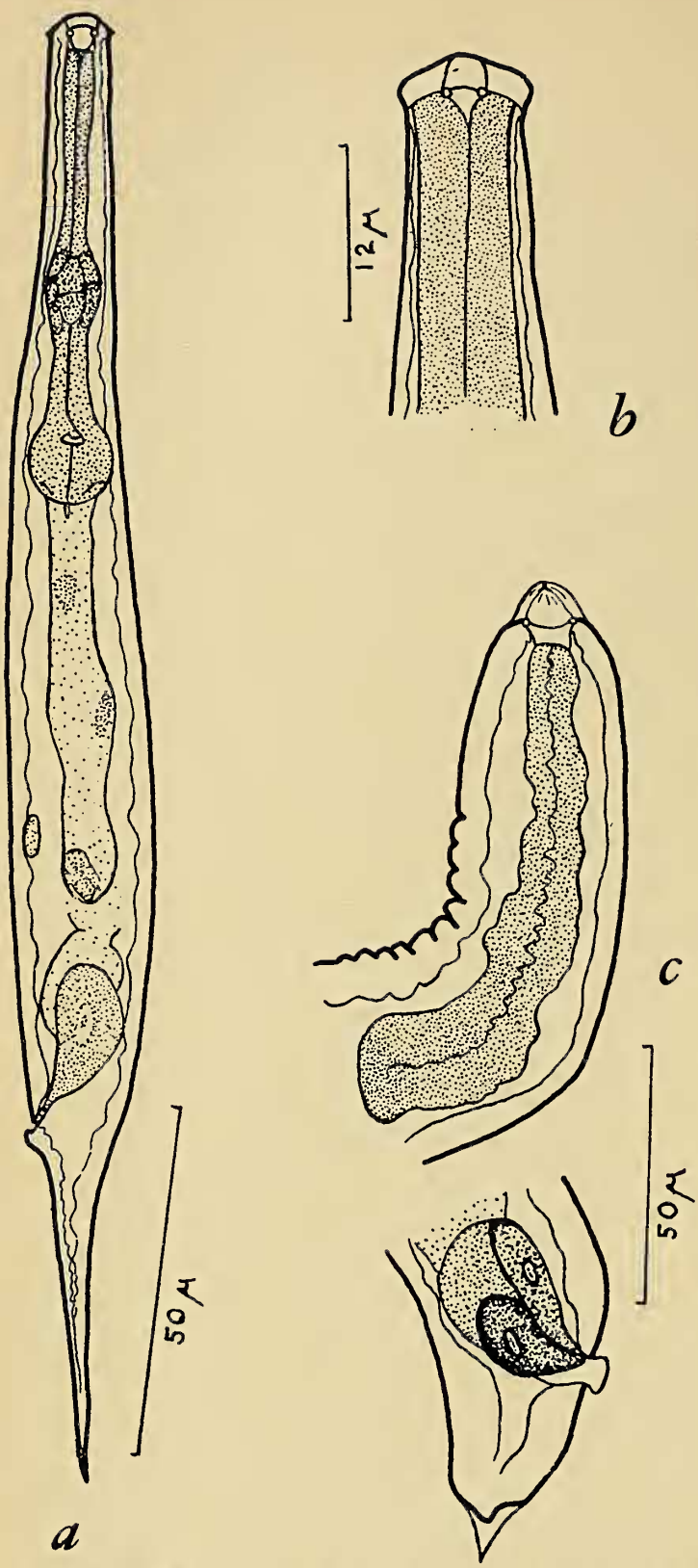


FIG. 7. *a*, First-stage larva; *b*, anterior end of late first-stage larva, *c*, first-stage larva in process of molting, showing loosened cuticle at anterior and posterior ends.

Fourth-stage larvae were first seen on the fifth day, although some third-stage larvae were still present until the seventh day. The reproductive organs developed rapidly during this period: the vulva of the female was apparent on the seventh day and the developing short spicule of the male on the ninth day.

A separate mesostom and protostom had begun to form by the thirteenth day. For most larvae the final molt occurred about the twenty-first day, and young adults were present on the twenty-third day. Embryonated eggs were first observed in the crop contents of a chicken on the thirty-second day. Egg laying apparently began between the thirtieth day, when the previous negative examination was made, and the thirty-second day.

## DESCRIPTION OF THE LARVAL STAGES

### *First-stage larvae*

Because of their small size and poor development the various structures of the first-stage larvae were discerned only with difficulty. The newly hatched larvae were long and slender, being somewhat attenuated at the anterior and posterior ends. The anterior end was rather blunt and the relatively long tail was acute. In the later period of this stage, the tail shortened considerably, the relative growth in width exceeded the growth in length, and the body assumed a more-or-less club-shaped appearance because of the enlargement of the several large rectal cells. The maximum size observed in this stage was a length of 339$\mu$ and a width of 24.9$\mu$. (See Table 5. Tables 5, 6, 7, and 8 show detailed measurements of a single typical larva for each of the days recorded. When size variations among the larvae on a particular day were significant, detailed measurements of more than one larva are given. When the sex of the larvae could be readily determined, measurements of both male and female larvae for a particular day were made.)

The cuticle was very thin and transparent and bore fine transverse striations. No papillae were apparent in this stage.

The oral opening was surrounded by a ring of highly refractile material. The transparent esophagus extended approximately one-third the length of the body and terminated as a large spherical bulb, possessing what appeared to be an esophageal valve. The intestine consisted of a number of rather large, ill-defined cells containing numerous large vacuoles of lipid-like material. The ventral anal

TABLE 5
MEASUREMENTS OF FIRST-STAGE LARVAE (ALL MEASUREMENTS IN MICRONS)

| MEASUREMENTS | DAYS AFTER INFECTION | | | | |
|---|---|---|---|---|---|
| | 3 | 5 | 8 | 17 | 18 |
| Length | 125 | 174 | 207 | 249 | 286 |
| Width (ant. end) | | 7 | 7 | 7.5 | 7 |
| Esophagus length | | 57.1 | | 107 | 93 |
| Anus to post. end | | 49.9 | 46.4 | 39.2 | 32.2 |
| Width (max.) | 14.3 | 17.8 | 17.8 | 32 | 18 |

aperture was often seen with a posterior cuticular fold partially covering it, and a few large rectal cells (apparently three in number) were well developed.

A nerve ring was seen encircling the esophagus at approximately two-thirds of the distance from its anterior end.

Neither an excretory pore nor an excretory cell was apparent in this stage.

A minute genital primordium, approximately 75 $\mu$ from the posterior end, was visible on the ventral body wall in several living specimens.

*Second-stage larvae*

Rapid growth occurred during this stage of larval development. The length increased from 990 $\mu$ in early second-stage larvae to 6.62 mm. in the latter portion of the stage. The larvae were long and slender with tapering tails, on the end of which four small papillae were discernible in the later part of the stage.

The cuticle was relatively thin and bore marked transverse striations. A provisional buccal capsule had begun to form, but no cephalic papillae were visible.

The esophagus was long and slender and in the late second stage extended approximately one-half the length of the body in some larvae. Two gland-like structures were apparent at its anterior end, and the esophageal-intestinal valve was well formed.

The intestine was a well-defined, thin-walled tube, varying only slightly in diameter and extending from the esophagus to the rectum. The cellular structure of its wall was apparent.

There were three large rectal cells and several smaller ones immediately anterior to the anus, which was located ventrally approximately 170–190 $\mu$ from the posterior end.

TABLE 6
MEASUREMENTS OF SECOND-STAGE LARVAE

| MEASUREMENTS | DAYS AFTER INFECTION | | | | |
|---|---|---|---|---|---|
| | 27 | 33 | 40 | 40 | 45 |
| Length | 999.6 | 1.85* | 2.6* | 4.5* | 6.62* |
| Width (ant. end) | 54 | 53.5 | 38.5 | 62 | 52 |
| Width (middle) | 67.8 | 89 | 107.8 | 170 | 172 |
| Width at anus | | 24 | 92.4 | 77 | 85 |
| Esophagus length | | 357 | 400 | 616 | 749 |
| Esophagus width (post. end) | | | | 43 | |
| Anus to post. end | 53.5 | 160 | 170 | 246 | 250 |
| Excretory pore to ant. end | | 178 | | 250 | 360.5 |
| Nerve ring to ant. end | | 89 | | 160 | 185.6 |
| Intestine width | | | 62 | 112 | |
| Genital primordia to post. end | | | | 890 | |

*These measurements in millimeters; all others in microns.

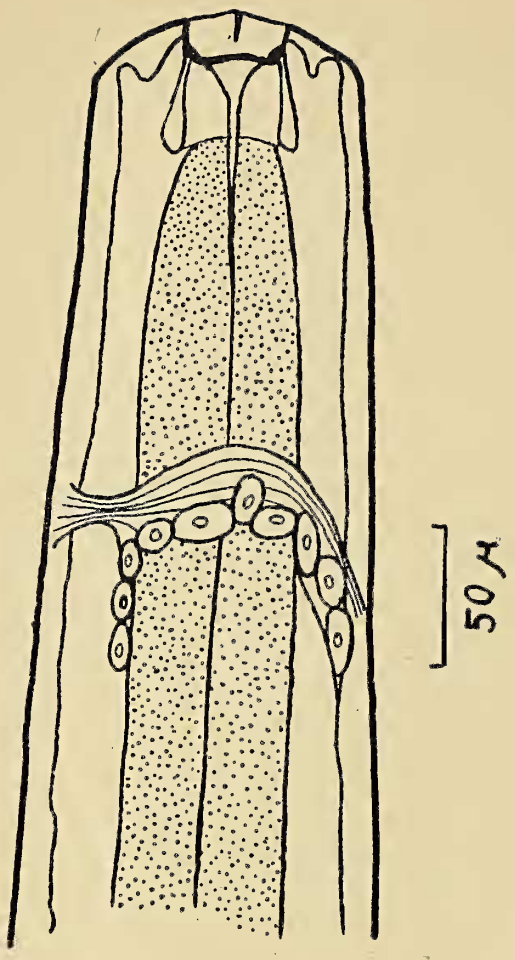


FIG. 8. Anterior and posterior ends of late second-stage larva.

*Third-stage larvae*

Third-stage larvae varied from 7.53 mm. to 8.31 mm. in length and had a maximum width of approximately 157 $\mu$. Other than in this feature, their over-all appearance was much the same as that of the second-stage larvae.

The cuticle was smooth and no striations were discernible, either transverse or longitudinal. The anterior oral opening was six-lobed, and four sublateral papillae were plainly visible projecting at right angles to the surface. No caudal papillae were present in this stage.

The esophageal gland-like structures apparent in the second stage were not discernible in the third-stage larvae, but a number of cells were seen to surround the esophagus at its anterior end. The esophagus was distinctly divided into an anterior muscular portion having a length of about 210 $\mu$ and a posterior glandular portion approximately 600 $\mu$ in length. The remainder of the alimentary tract appeared much the same as that of the second-stage larvae.

TABLE 7
MEASUREMENTS OF THIRD-STAGE LARVAE*

| | |
|---|---|
| Days after infection | 51* |
| Length† | 7.53–8.31 |
| Width (ant. end) | 53.5 |
| Width (max.) | 157 |
| Width (at anus) | 92.3 |
| Buccal capsule length | 35.7 |
| Esophagus length | 846 |
| Esophagus width (post. end) | 71.5 |
| Intestine width (max.) | 107 |
| Nerve ring from ant. end | 178.5 |
| Excretory pore from ant. end | 303 |
| Anus to post. end | 184 |

*In intermediate host. For measurements of third-stage in definitive host, see Table 7.

†This measurement in millimeters; all others in microns.

An excretory pore and a large excretory cell were plainly visible on the ventral side of the esophagus, 250–300 $\mu$ from the anterior end.

The nerve ring and several small ganglion cells encircled the esophagus, about 100 $\mu$ anterior to the excretory pore.

The genital primordium was visible in the living larvae ventrad of the intestine, approximately 500 $\mu$ from the anus.

In addition to the excretory pore and cell apparent in the second stage, two lateral excretory canals were traced from the region anterior of the excretory pore to very near the tip of the tail. Each canal sent off an anterior branch which joined ventrad of the esophagus and entered the excretory cell as a common excretory duct. The lateral canals

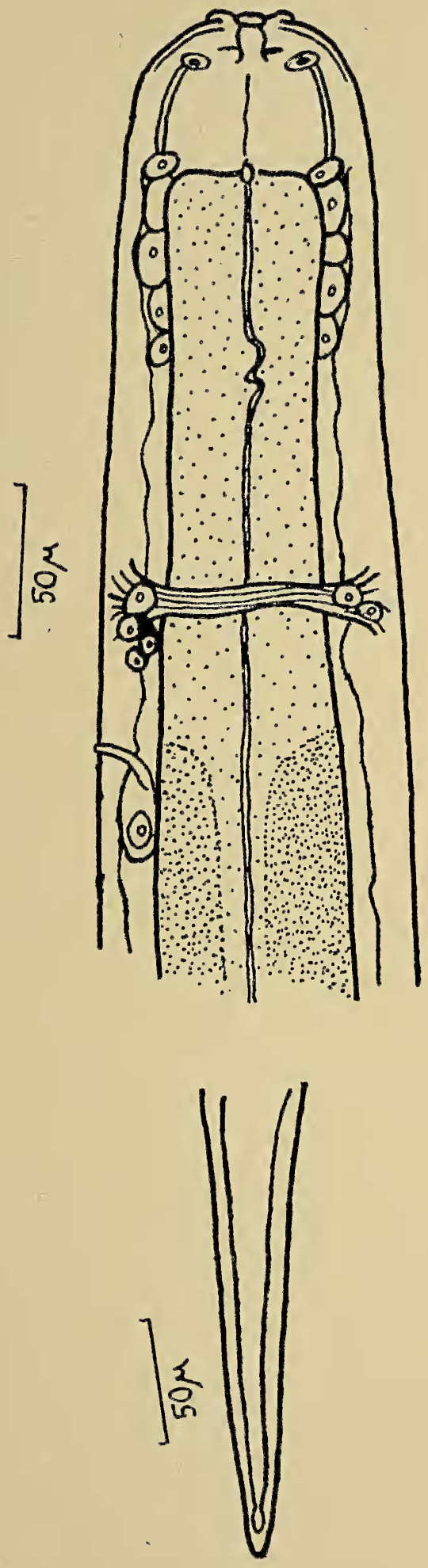


FIG. 9. Anterior and posterior ends of third-stage larva.

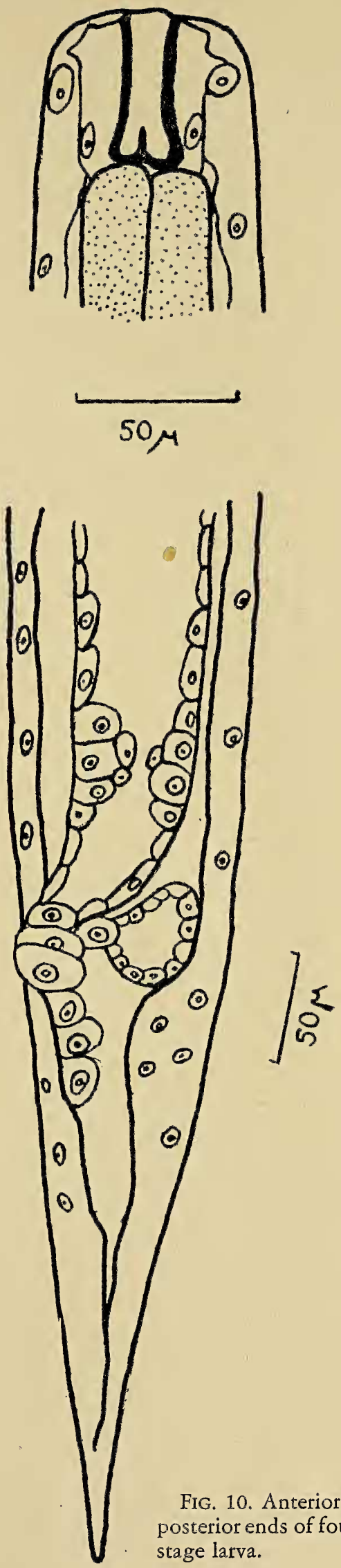


FIG. 10. Anterior and posterior ends of fourth-stage larva.

TABLE 8

MEASUREMENTS OF THIRD- AND FOURTH-STAGE LARVAE IN THE DEFINITIVE HOST

| MEASUREMENTS | DAYS AFTER INFECTION | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | THIRD STAGE | | FOURTH STAGE | | | | | | | | | | | | |
| | 2 | 4 | 5 | 5 | 7 | 9 | 11 | 11 | 13 | 16 | 18 | 18 | 21 | 21 | 23 |
| | — | — | ♀ | ♂ | ♀ | ♀ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♀ | ♀ |
| Length* | 8.23 | 10.38 | 10.38 | 10 | 12.08 | 10.1 | 10.3 | 9.7 | 12.1 | 11.4 | 12.6 | 10.4 | 12.3 | 14 | 12.8 |
| Width (middle) | 257 | 250 | 222 | 176 | 250 | 214 | 303 | 267 | 267 | 326 | 307 | 284 | 293 | 290 | 310 |
| Width at vulva | | | | | | | 176 | | 183 | | 185 | | 204 | 196.5 | 196 |
| Width at anus | 89.4 | 87 | 89.4 | 92.8 | 100 | 87 | 92.8 | 103 | 104 | 100 | 100 | 110 | 100 | 92.8 | 92.8 |
| Oral opening width | 35.7 | 35.7 | 32 | 32 | 35.7 | 40 | 32 | 28.6 | 32 | 35.7 | 24 | 21.4 | 21.4 | 28.6 | 35.7 |
| Buccal capsule length | 42.9 | 54 | 42.9 | 50 | 47 | 50 | 50 | 42.9 | 47 | 42.9 | 45 | 40 | 57.2 | 57.2 | 35.7 |
| Esophagus length (ant. portion) | | 340 | 250 | 330 | 330 | 287 | 267 | 330 | 287 | 267 | 250 | 267 | 293 | 304 | 271 |
| Esophagus length (total) | 930 | 1,285 | 1,039 | 1,150 | 1,212 | 1,032 | 1,140 | 1,080 | 1,230 | 1,060 | 1,039 | 1,140 | 1,220 | 1,230 | 1,054 |
| Esophagus width (post. end) | 62 | 77 | 85 | 100 | 92.8 | 92.8 | 85 | 85 | 92.8 | 100 | 92.8 | 92.8 | 96.5 | 92.8 | 103.5 |
| Nerve ring to ant. end | 250 | 268 | 214 | 287 | 287 | 234 | 228 | 275 | 234 | 172 | 226 | 214 | 222 | 279 | 214 |
| Excretory pore to ant. end | 357 | 392 | 303 | 303 | 392 | 303 | 326 | 360 | 357 | 330 | 342 | 307 | 357 | 379 | 307 |
| Intestinal width (max.) | 89.4 | 71.4 | 89.4 | 77 | 77 | 75 | 96 | 96 | 81 | 73 | 54 | 68 | 74 | 71 | 71.4 |
| Vulva to post. end. | | | | | 1,130 | 850 | 784 | | 950 | | 950 | | 910 | 883 | 1,000 |
| Anus to post. end. | 340 | 357 | 340 | 392 | 430 | 330 | 357 | 392 | 370 | 330 | 370 | 340 | 340 | 360 | 392 |

*These measurements in millimeters; all others in microns.

were each embedded in a multinucleate lateral line 50 $\mu$ in width.

The nervous system appeared much the same as in the second-stage larvae.

The sexes could be distinguished readily in the third stage. In the female the genital primordium could be seen attached to the ventral body wall approximately 650 $\mu$ from the posterior end. In the male the primordium was similarly situated but unattached. The primordium in both sexes measured approximately 35 $\mu$ by 14 $\mu$ in this stage and was composed of several cells.

*Fourth-stage larvae*

In general proportions the fourth-stage larvae differed little in appearance from those of the third stage. Early fourth-stage larvae measured about 10 mm. in length while those in the later portion of the stage measured from 12.8 to 14 mm.

Fine transverse striations were evident in the transparent cuticle. The four sublateral papillae each measured approximately 4.6 $\mu$ in length. In addition, by the twenty-first day, the two latero-caudal papillae had begun to form, and the preanal and postanal papillae of the males were evident.

By the thirteenth day the separate protostom and mesostom could be differentiated, and in the later part of the stage the buccal capsule appeared much the same as in the adult.

The remainder of the alimentary tract showed little change from that of the third-stage larvae, the intestine being displaced somewhat to allow room for the developing reproductive organs.

The lateral excretory canals were seen to terminate in the latero-caudal papillae in the late fourth-stage larvae. Other than this, the excretory system had apparently achieved its full development by the end of the third stage.

The only additional development noted in the nervous system in the fourth stage was the appearance of two large dorsal and two large ventral ganglion cells near the nerve ring.

In the fourth-stage larvae the greatest development occurred in the reproductive system. By the fourth day the genital primordium in both sexes measured approximately 214 $\mu$ in length and in the case of the female was attached to the ventral body wall. The point of attachment, which later became the vulva, was approximately 894 $\mu$ from the posterior end. In the male the genital primordium became a U-shaped tube with two terminal bulbous growing tips developing toward the posterior end. By the ninth day the short spicule of the male had begun to form and the vulva of the female was apparent, although it remained closed externally during the entire fourth stage. The reproductive tract, at least in the female, appeared almost complete by the thirteenth day. By the sixteenth day the long spicule of the male could be recognized. The male reproductive system was well formed by the twenty-first day; the ejaculatory duct could be seen to enter the ventral side of the rectum at its posterior terminus, the anal papillae were evident, and both spicules were well developed.

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